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RESEARCH ARTICLE

Tendon material properties vary and are interdependent among turkey hindlimb muscles

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SUMMARY

The material properties of a tendon affect its ability to store and return elastic energy, resist damage, provide mechanical feedback and amplify or attenuate muscle power. While the structural properties of a tendon are known to respond to a variety of stimuli, the extent to which material properties vary among individual muscles remains unclear. We studied the tendons of six different muscles in the hindlimb of Eastern wild turkeys to determine whether there was variation in elastic modulus, ultimate tensile strength and resilience. A hydraulic testing machine was used to measure tendon force during quasi-static lengthening, and a stress–strain curve was constructed. There was substantial variation in tendon material properties among different muscles. Average elastic modulus differed significantly between some tendons, and values for the six different tendons varied nearly twofold, from 829±140 to 1479±106 MPa. Tendons were stretched to failure, and the stress at failure, or ultimate tensile stress, was taken as a lower-limit estimate of tendon strength. Breaking tests for four of the tendons revealed significant variation in ultimate tensile stress, ranging from 66.83±14.34 to 112.37±9.39 MPa. Resilience, or the fraction of energy returned in cyclic length changes was generally high, and one of the four tendons tested was significantly different in resilience from the other tendons (range: 90.65±0.83 to 94.02±0.71%). An analysis of correlation between material properties revealed a positive relationship between ultimate tensile strength and elastic modulus (r^2=0.79). Specifically, stiffer tendons were stronger, and we suggest that this correlation results from a constrained value of breaking strain, which did not vary significantly among tendons. This finding suggests an interdependence of material properties that may have a structural basis and may explain some adaptive responses observed in studies of tendon plasticity.

Key words: tendon, tissue material properties, elasticity, tensile strength.

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INTRODUCTION

Tendons transmit force between muscles and the skeleton, but they also play an important role as springs (Cavagna et al., 1977; Alexander, 2003). Elastic deformation of tendons allows them to store potential energy, provide mechanical feedback and amplify or attenuate muscle power (Roberts, 2002; Konow et al., 2011; Roberts and Azizi, 2011). In walking and running, the stretch and recoil of tendons improves locomotor economy by reducing muscle work and allowing muscles to operate at lengths and velocities that are favorable for force production (Heglund et al., 1982; Alexander, 1991; Roberts et al., 1997; Lichtwark and Wilson, 2006; Sawicki et al., 2009).

The mechanical properties of any given tendon will determine its tendency to stretch, shorten and potentially fail under muscular loads. These mechanical properties in turn affect muscle length changes, joint motions and the cycling of elastic strain energy. Presumably, some matching of tendon properties to muscle properties allows for both effective spring-like activity as well as a degree of protection against tendon damage or failure (Ker et al., 1988). Variation in tendon dimensions is an important means of tuning mechanical properties. For instance, if material properties are held constant, a short tendon with a large cross-sectional area will tend to be stiffer than a long thin tendon.

It is less clear to what extent the material properties of tendons vary in response to functional demands. It is commonly stated that all vertebrate tendons are relatively similar in elastic modulus (EM) (Zajac, 1989; Alexander, 2003), and some comparative studies have supported this assumption (Bennett et al., 1986; Pollock and Shadwick, 1994). However, reported values vary widely, from low EM values of 160 MPa in juvenile pig tendon (Shadwick, 1990), to values exceeding 2000 MPa for human tendon (Maganaris et al., 2004). It is difficult to interpret the variation in material properties from literature values because methodological challenges associated with these measurements leave some uncertainty as to whether variation in reported values might be explained, in part, by differences in measurement techniques. One particular challenge for *in vitro* measurements is the technique for clamping soft tissue, which can lead to errors in strain measurements due to slipping, as well as underestimates of tendon strength due to damage at the tendon–clamp interface. Recent studies have avoided problems associated with clamp slipping by using surface markers in an intact *in situ* preparation, and have demonstrated variation in EM both between tendons of different muscles (Cui et al., 2009) and along the length of an individual tendon (Arruda et al., 2006; Wood et al., 2011).

We used wild turkey hindlimb muscles to determine whether tendon material properties vary among muscles. Turkeys are a good model because their distal hindlimbs include numerous muscles with long free tendons. Most importantly, many of their tendons are continuous with ossified regions that provide a means for secure

clamping, avoiding many of the problems associated with clamping soft tissues. By isolating six different muscle–tendon units (MTUs) from the same limbs, we were able to achieve a broad sample for comparisons of tendon EM, strength and resilience. Our primary goal was to determine whether these properties varied among tendons. A secondary goal was to determine whether differences in one property correlated with differences in another.

MATERIALS AND METHODS

For tendon testing, data were collected from hindlimb tendons of captive-bred wild turkeys (*Meleagris gallopavo* Linnaeus 1758; *N*=14; mass: 3.1–4.0 kg). Six different hindlimb muscles were identified following the study of Harvey and co-workers (Harvey et al., 1968). The tendons studied belonged to the following muscles: gastrocnemius complex (GA; *N*=8), tibialis cranialis (TC; *N*=9), extensor digitorum longus (EDL; *N*=12), flexor perforans digiti 3 (FPD3; *N*=12), flexor perforans et perforatus digiti 3 (FPPD3; *N*=12) and flexor hallucis longus (FHL; *N*=12) [nomenclature follows Vanden Berge and Zweers (Vanden Berge and Zweers, 1993)]. Tendon samples were harvested from frozen specimens used in previous studies. Samples were excluded if they were damaged during dissection or preparation. Dimensional data for the associated muscles were collected from eight separate individuals of comparable weight (3.1–3.6 kg). All animal use was approved by the Brown University Institutional Animal Care and Use Committee.

Sample preparation

All tendon samples were isolated from intact hindlimbs stored at –18°C prior to testing. Before dissection, limbs were thawed at room temperature in zip-seal bags to avoid desiccation. During dissection, each MTU was isolated from the origin of the muscle to the distal insertion of the tendon using blunt dissection. The free tendons, including both bony and soft regions, were separated from their muscle bellies and placed in saline at room temperature. When the dissection was complete, tendons were wrapped in saline-saturated gauze and stored at –18°C, and thawed again on the day of mechanical testing. One to two repeated freezing and thawing cycles, as in the case of our study, have limited effect on tendon EM (Ker, 1981; Chen et al., 2011), whereas multiple freeze–thaw cycles may damage tendon microstructure (Chen et al., 2011).

Tendons were isolated from hindlimbs of animals that had been used previously in locomotor studies. There was some variation among individuals in the extent of treadmill training they had experienced, and the details of the locomotor regime. Some animals were trained for uphill running, others for downhill, and others received minimal training. These differences were controlled for statistically as described below.

Morphometrics

Muscle dissection and measurement were performed before tendon testing on separate animals. Each muscle was weighed and then longitudinally bisected to facilitate measurements of fascicle length and pennation angle (Roberts et al., 1998). Average muscle physiological cross-sectional area (A_m) was calculated as:

$$A_m = (M_m \cos\theta)/(\rho_m L_m), \quad (1)$$

where M_m is the mass (g) of the muscle, θ is the average measured pennation angle (deg) of the muscle fibres, ρ_m is the density of muscle, assumed to be 1.06 g cm^{-3} (Mendez and Keys, 1960), and L_m is the average measured fascicle length (cm).

The digital flexors and extensors all included a region of soft tendon bounded at both the proximal and distal ends by segments of bony tendon. For these tendons resting length (L_0) was defined as the distance from the proximal bony–soft tendon junction to the distal bony–soft tendon junction (Fig. 1). L_0 was measured before mechanical measurements while the tendon was clamped at a passive tension of 0.8 N. For the TC and GA, L_0 was determined from the distance between the marked segment of interest as determined from video. The mass of each soft tendon was obtained after mechanical measurements. Average tendon cross-sectional area (A_t) was calculated from tendon volume and length:

$$A_t = M_t/(\rho_t L_0), \quad (2)$$

where M_t is tendon mass in grams and ρ_t is the density of tendon [1.12 g cm^{-3} (Ker, 1981)].

Mechanical measurements

We used a hydraulic testing machine (858 Mini-bionix II, MTS Systems, Eden Prairie, MN, USA) operating under length control to measure tendon stiffness, resilience and breaking strength. For the four digital flexor and extensor tendons, the calcified regions of the tendon provided a very favorable site for clamping, as it was possible to clamp bony tendon very firmly without significantly damaging the tissue. Tendons were trimmed to leave approximately 1 cm of calcified tendon, which was free of any overlying soft tissue and which allowed for clamping while preserving the bony–soft tendon junction (Fig. 1). Digital flexor tendons in the turkey hindlimb all follow the same pattern of bony and soft regions. Tendons are calcified at the interface with the muscle, then soft as they cross the intertarsal (ankle) joint, followed by a bony region that runs the length of the tarsometatarsus. Distal to the tarsometatarsus, the tendons become soft again as they cross joints of the digits, and many interconnect *via* vinculae. In mature tendons, the transition from bony to soft is an abrupt one; there does not appear to be a significant region of transitional tissue. We used the proximal bony region, at the muscle, and the bony region within the tarsometatarsus to clamp our digital tendon samples, thus measuring the soft tendon region that spanned the intertarsal joint. The proximal end of the tendon was clamped to the top clamp, the controllable, mobile component of the MTS machine. The distal bony region was clamped to a stationary clamp attached to the MTS force transducer. Sandpaper was used to increase clamp friction in both clamps, and the surface of the bony tendon was scraped with a razor blade to ensure that there was no overlying soft tissue. Tendons were irrigated with physiological saline and kept wrapped in a saline-saturated cloth in between data collections to avoid desiccation.

The GA and TC tendons required a different clamping technique. For GA, the proximal end divides into two as the bony tendons follow the myotendinous junctions of the lateral and medial gastrocnemius bellies. Both divisions were clamped in the upper, mobile clamp. The distal ends of both GA and TC are soft and insert directly into the tarsometatarsal bone. Dissection of these tendons involved excision of approximately 1 cm^3 blocks of bone at the distal insertions of these tendons onto the tarsometatarsus. The distal bony block containing the tendon insertion was potted in a liquid plastic compound (Smooth-Cast 300Q, Smooth-On, Easton, PA, USA) and this complex was clamped to the bottom, stationary clamp. L_0 was defined from the proximal bony–soft junction to the point of tendon insertion into the bone.

Force and length signals were sampled at a frequency of 1024 Hz *via* MTS Station Manager software. The same hardware and software system was used to control position of the top clamp within the MTS frame. Prior to measurements, clamp position was adjusted

Fig. 1. Photograph of a sample tendon from the hindlimb of the wild turkey *Meleagris gallopavo*. Arrowheads indicate the bony–soft tendon junction. Scale bar, 1 cm.

to achieve a resting tension of 0.8 N, a force that corresponded to no visible slack in the tendon. Each measurement trial consisted of a series of 200 cycles of sinusoidal stretch–shortening excursions at 4 Hz, followed by a linear, isovelocity ramp lengthening. A frequency of 4 Hz was chosen as it is close to the loading frequency *in vivo* during fast running. The sine wave amplitude was set to yield a 4% L_0 excursion for each tendon. The sine wave cycles preconditioned the tendon to avoid changes in EM that are known to occur with initial load cycles in isolated tendons (Schatzmann et al., 1998). Over the last 10 cycles, resilience was calculated as the average proportion of energy conserved between each lengthening–shortening cycle. The ramp lengthening following the sine wave cycles was driven at a rate of 1 mm s^{-1} for all specimens. Because tendon lengths varied, this loading rate did not result in identical strain rates for individual tendons, but this slight variation was highly unlikely to affect our measurements as tendon modulus is insensitive to loading rate across a broad range of values (Ker, 1981). Measurements taken during the ramp lengthenings were used to calculate strain (Fig. 2A), stress (Fig. 2B) and continuous EM (Fig. 2C).

The stress at the point of complete failure of the tendon, as indicated by a precipitous drop to zero force (Fig. 2B) and visible tendon rupture, was taken as the ultimate tensile strength (UTS). It should be noted that this estimate may be lower than the failure strength *in vivo*, as it is impossible to ensure that force is evenly distributed across all tendon fibrils in a clamped tendon (Ker, 2007).

Because of the potential for end-effects to distort mechanical data from clamping of short, thick tendons (Legerlotz et al., 2010), and a suspicion of slipping in GA and TC based on our observations, MTS data were not used to determine length for GA and TC. Instead, strain was determined from surface markers tracked by high-speed video. Points used for digitizing were either small surface markers (Reptile Sand, Zoo Med, San Luis Ospina, CA, USA) or pins (000 Insect Pins, Austerlitz, Czech Republic). Surface markers were glued onto the soft tendon at 5 mm intervals along the length of the tendon, and pins were inserted through the tendon transversely in a similar distribution. Marker positions were digitized using a DLT Data Viewer (Hedrick, 2008), then smoothed for high-frequency noise using a quintic spline interpolation (s.d.=0.01–0.1). Digitized length changes between the most proximal and distal of the soft tendon markers were used to calculate strain.

One set of high-speed video measurements was also made for two digital tendons to verify that MTS measurements accurately tracked tendon strain. Strain measured from video was within 3% of MTS measurements, confirming that end-effects and slipping were minimal for these tendons. Therefore, MTS length measurements were used to measure length and to calculate strain for the digital tendons.

Data analysis

Data collected by MTS Station Manager software was imported into IGOR Pro 6.0 (Wavemetrics, Lake Oswego, OR, USA), and a force–length curve was constructed for each tendon. Strain (ε) was

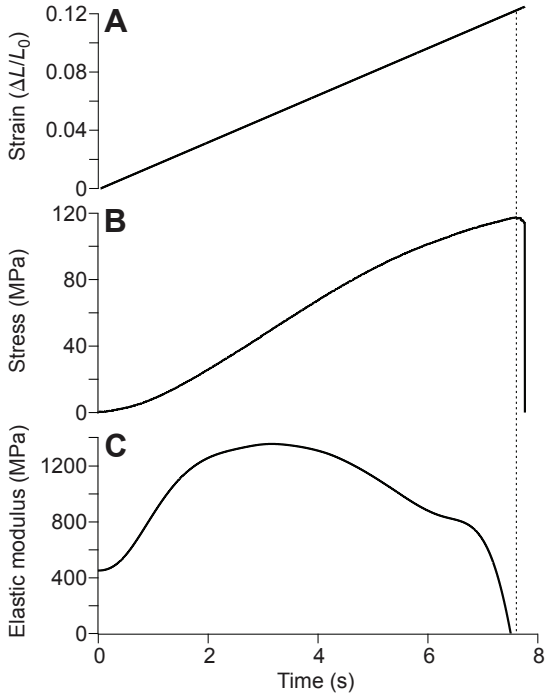

Fig. 2. Sample strain (A) and stress (B) measurements for a turkey FPPD3 tendon. Data are for a controlled isovelocity lengthening at 1 mm s^{-1}. Continuous elastic modulus (EM; C) was measured as the derivative of stress over strain. Maximum EM was determined by taking the peak of the continuous EM curve (C). Breaking stress and strain were taken at the point of maximum stress, as indicated by the dotted line.

defined as $\Delta L/L_0$ using MTS data for the digital tendons and digitized data for GA and TC. Stress was calculated as:

$$\sigma = F/A_t, \quad (3)$$

where σ is stress (MPa), F is force (N) and A_t is the average cross-sectional area of the tendon (mm^2).

EM was measured as the instantaneous rate of change in stress over change in strain:

$$EM = \Delta\sigma/\Delta\varepsilon. \quad (4)$$

To ensure that maximum EM was measured for each tendon, the derivative of the stress–strain curve was graphed and the maximum was identified. All values are presented as means ± 1 s.d. To test the hypothesis that the tendons from different muscles differ with respect to their material properties, we ran a multi-factorial ANOVA with EM, resilience and UTS as dependent variables, and tendon as an independent factor, and we used a mixed-model design to control for individual and training effects. We used Tukey-corrected *post hoc* tests to establish whether there were differences between the EM of different tendons and to reduce the risk of type I errors in multiple comparisons.

RESULTS

All tendons showed a J-shaped relationship between stress and strain (Fig. 3A), but the stress–strain relationship differed among tendons of different muscles. Fig. 3A shows the variability in stress–strain curves for six different tendons from a single animal. For all tendons, continuous EM reached a plateau over a region of approximately 3–6% strain (Fig. 3B). This plateau region indicates the linear portion

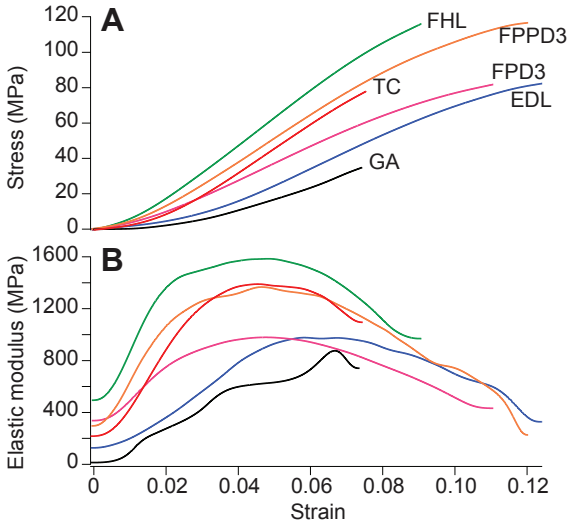

Fig. 3. Stress–strain relationships (A) and continuous elastic moduli (B) for six different tendons in a single turkey hindlimb specimen. Colors in B are as labelled in (A).

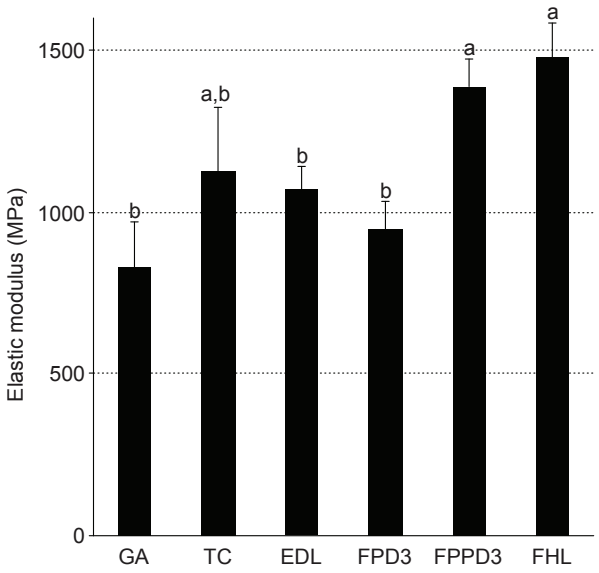

Fig. 4. Mean EM values for all tendons measured. Error bars show standard deviation (N≥8). Values marked 'a' differ from those marked 'b' (Tukey-corrected ANOVA, all P<0.05). TC (marked 'a,b') does not differ from any other averages (Tukey-corrected ANOVA, P>0.05).

of the stress–strain curve, which is bound by the toe region at lower strains and a region of yield at higher strains.

Maximum EM varied among tendons (Fig. 4, Table 1). FHL had the highest average EM value (1479±106 MPa) and GA had the lowest (829±140 MPa). Statistical comparisons between average maximum EM values showed differences when comparing both FHL and FPPD3 with EDL, FPD3 or GA (Tukey-corrected ANOVA; all P<0.05). All other comparisons between averages of maximum EM values were not significant.

Resilience was measured only for the four digital tendons where bony tendon segments at both ends allowed for secure clamping. Resilience measurements for these tendons showed a range of 90.65–94.02% (Table 1). The only digital extensor tested, EDL, had the lowest resilience value (90.65±0.83%), which was significantly different from the three digital flexor tendons, FPD3, FPPD3 and FHL (Tukey-corrected ANOVA, all P<0.05). All digital flexor tendons had similar resilience values.

Measurements of ultimate tensile strength (UTS) were also obtained for the four digital tendons (Fig. 5, Table 1). Due to challenges associated with clamp slipping and damage at high forces, not all GA and TC specimens were successfully stressed to failure, and these tendons were thus omitted from our comparative analysis of strength. FHL and FPPD3 were stronger than both EDL (ANOVA, P<0.05 and P<0.01, respectively) and FPD3 (ANOVA, P<0.05 and P<0.01, respectively). To determine if variation in tendon strength was related to tendon stresses *in vivo*, we used muscle and tendon dimensions to calculate maximum stress-in-life, assuming peak muscle stress of 300 kPa. All UTS measurements were higher than calculated maximum stress-in-life values (Fig. 5).

A clear relationship that emerged was a positive correlation between UTS and EM (Fig. 6A). On average, more compliant tendons failed at lower stresses. This correlation was present whether analyzed by individual tendon trial (N=45, r^2=0.79, P<0.0001) or for global averages for each tendon (N=4, r^2=0.92). Individual measurements for EDL and FPD3 clustered at low EM and low UTS, while individual FHL and FPPD3 measurements clustered at high EM and high UTS.

By contrast, there was no significant relationship between strain at failure and EM (Fig. 6B). Digital tendons generally failed at a strain of approximately 10% L_0, and there was no correlation between failure strain and EM for individuals (N=45, r^2=0.04, P=0.215), or tendon averages (N=4, r^2=0.17). Additionally, no differences were found between any of the four average failure strain values.

DISCUSSION

We found significant variation among different tendons in EM, resilience and UTS. These results add to an increasing body of evidence indicating that tendon is a dynamic tissue with the potential

Table 1. Mean values for mechanical and dimensional measurements

Tendon	Elastic modulus (MPa)	Ultimate tensil strength (MPa)	Maximum strain (% L_0)	Resilience (%)	Muscle mass (g)	Tendon length (mm)	Tendon area (mm^2)
GA	829±140	–	–	–	46.28	42±9	19.29±1.61
TC	1124±197	–	–	–	16.62	27±3	6.73±0.81
EDL	1072±67	68.22±13.83	9.30±1.91	90.65±0.83	4.10	42±3	2.59±0.56
FPD3	948±85	66.83±14.34	9.16±1.64	93.77±0.64	5.66	59±5	3.34±0.80
FPPD3	1390±82	112.37±9.39	10.67±1.20	94.02±0.71	9.67	62±5	1.70±0.28
FHL	1479±106	107.25±14.12	9.14±1.01	93.29±0.48	7.79	56±2	2.39±0.37

Measurements are means ± s.d.

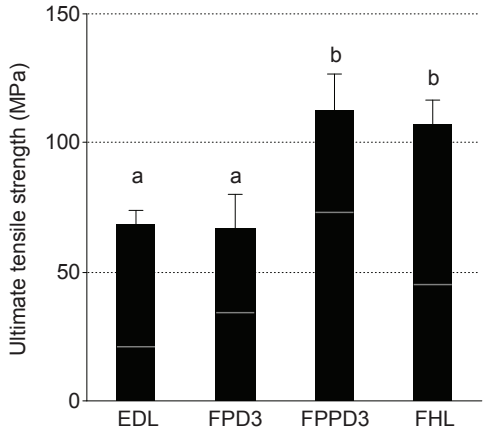

Fig. 5. Average ultimate tensile strength values of four digital tendons ($N \geq 11$). Averages marked 'a' differ significantly from averages marked 'b' (Tukey-corrected ANOVA, all $P<0.05$). Thin gray lines within the bars indicate the estimated maximum stress-in-life, calculated from muscle dimensions and an assumed maximum muscle stress of 300 kPa.

to modulate material properties in response to mechanical demand. Our work also lends support to the hypothesis that some material properties are interdependent. Specifically, we find a correlation between EM and UTS, such that stiffer tendons are also stronger.

Variation in tendon material properties

Literature EM values for vertebrate tendon vary by an order of magnitude, from less than 200 MPa (Shadwick, 1990) to greater than 2000 MPa (Maganaris et al., 2004), with a wide range of values in between (Bennett et al., 1986; Bennett and Stafford, 1988; Shadwick, 1990; Lieber et al., 1991; Pollock and Shadwick, 1994; Maganaris and Paul, 2002; Cui et al., 2009). Some of this variation is probably explained by real variation in tendon properties. For example, the very low value of 160 MPa reported for juvenile pig tendons is consistent with the observation that developing tendons appear to be more compliant than those of adults (Shadwick, 1990). However, it is difficult to draw conclusions about variation in EM from literature measurements due to variation in measurement methods and materials. Tendon tensile tests are methodologically simple but may involve several sources of variation, notably including clamp slipping, but also in the methods used to determine cross-sectional area and prepare samples (Woo, 1982; Svendsen and Thomson, 1984; Legerlotz et al., 2010). Studies that use identical techniques for several different tendons can provide reliable insight into the extent of variation in tendon material properties. Cui and co-workers (Cui et al., 2009) used *in situ* measurements to demonstrate variation in EM among tendons of three cat hindimb muscles, the tibialis cranialis (528 MPa), gastrocnemius (516 MPa) and extensor digitorum longus (969 MPa). Recent studies also present evidence that EM varies within a tendon, with a longitudinal gradient of stiff nearer the bone and more compliant nearer the muscle (Wood et al., 2011). The variation in EM among different turkey hindlimb muscles observed in the present study lends further support to the idea that tendon modulus varies.

Our measured resilience values for the tendons of turkey hindlimb muscles fell within the range of previously reported values. Studies that evaluate *in vitro* tendon resilience across a broad spectrum of mammals have found values within the range of 86–97% (Bennett et al., 1986; Pollock and Shadwick, 1994). The observed pattern in the present study was that EDL, the lone digital extensor, was less

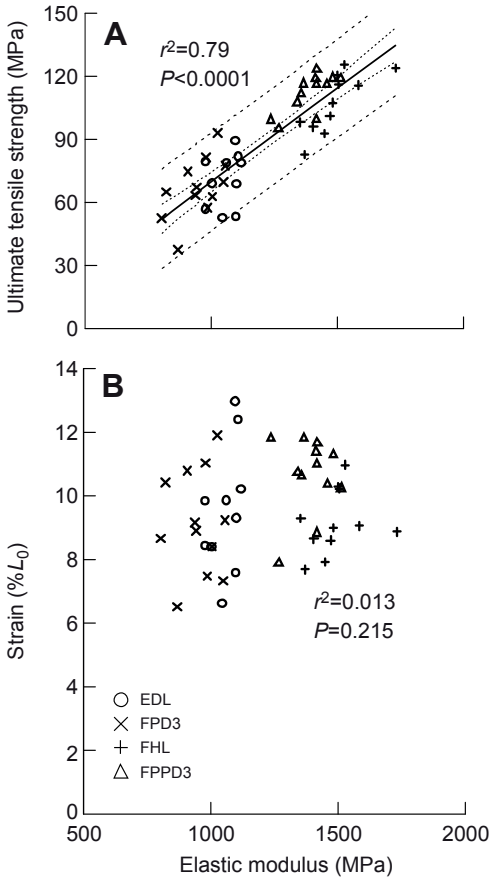

Fig. 6. Relationship between EM and ultimate tensile strength (A), and between EM and failure strain (B) for four different types of digital tendons. For each individual tendon stretched to failure, the measured stress (A) and strain (B) at which the tendon broke is plotted against the EM of the tendon. In A, the fine dotted lines show quartile confidence limits and the thick dotted lines show the prediction limits from least-squares regression.

resilient than the three digital flexors, while none of the digital flexors differed from each other (Table 1). This trend is consistent with that observed in mature pigs, where resilience was lower in the digital extensors compared with the digital flexors [82.5 *vs* 90.8% (Shadwick, 1990)]. When measured by ultrasound *in vivo*, human tendon resilience values tend to be lower than results from *in vitro* studies [e.g. 78% (Kubo et al., 2002), 82% (Maganaris and Paul, 2002) and 81% (Maganaris and Paul, 2000)]. Maganaris (Maganaris, 2002) highlights advantages of the *in vivo* method in comparison with isolated measurements such as avoidance of potential artifact due to clamping and tendon freezing, and the ability to use muscle contraction to load tendon. However, ultrasound techniques present difficulties in defining the point of 0% strain and inaccuracies attributable to the two-dimensional interpretation of three-dimensional movement, factors that may limit the accuracy of *in vivo* resilience measurements (Maganaris, 2002; Magnusson et al., 2008).

Measured values of UTS were also variable among tendons of different turkey hindlimb muscles and within the range of values reported previously for mammals and birds. We found a range in average breaking stress of 66.83–112.07 MPa among the four digital tendons, with FHL and FPPD3 breaking at significantly higher stress than FPD3 and EDL. Our values are within the range measured previously for turkey tendons [54–117 MPa (Bennett and Stafford,

1988)], mammal tendons [62–107 MPa (Bennett et al., 1986) and 40–90 MPa (Shadwick, 1990)] and turkey aponeurosis [53 MPa along the longitudinal axis (Azizi et al., 2009)].

It has been suggested that UTS values should be interpreted as a lower limit, as challenges associated with clamp slippage at high forces or tendon damage at the site of clamping can lead to underestimates of breaking force (Bennett et al., 1986). While clamping bony tendon segments and using video to measure tendon strain allowed us to avoid problems associated with stress concentrations at the clamps and slipping, our estimates of ultimate tensile strength are still probably subject to problems of uneven distribution of force across fibrils. The obvious yield region of the stress–strain curves (Figs 2, 3) may be explained in part by the sequential failure of many independent fibrils. Although we made efforts to ensure that our clamping oriented the bony and soft tendon longitudinally, it is possible that tendon alignment in our preparations increased the chance of uneven stress concentrations transversely across the tendon. Thus our estimates of UTS should be taken as a lower limit for the failure stress of tendon *in vivo*.

Relationship between stiffness and strength

We found a positive correlation between UTS and EM across the range of tendons measured. By contrast, there was no significant relationship between EM and breaking strain. There was variability among different samples, but on average all samples broke at a strain of close to 10% L_0. We hypothesize that the correlation between material strength and stiffness results from a relatively constrained value of breaking strain. If a tendon can stretch by only a certain strain before failure, then differences in EM must necessarily be associated with differences in breaking strength. If this is the case, then more compliant tendons will also be weaker. This positive correlation between stiffness and strength has been observed previously among avian tendons (Bennett, 1995). It has also been demonstrated in an analysis that included both calcified and uncalcified turkey tendons: calcified tendons are approximately 10 times stiffer than non-calcified tendons, and also break at higher stresses (Bennett and Stafford, 1988).

Support for a hypothesized mechanistic link between material stiffness and strength comes from both interventional and comparative studies. Limb-immobilized rats show a decrease in both material strength and material stiffness when compared with controls (Matsumoto et al., 2003). Patterns of change in tendon material properties with ontogeny and ageing also tend to support the idea that EM and UTS are coupled (reviewed in Narici et al., 2008). The tendons of young pigs are more compliant and weaker compared with those of mature pigs (Shadwick, 1990). A study of rabbit Achilles tendon showed that immature animals have low stiffness (281 MPa) and strength (23.9 MPa) compared with mature animals (stiffness 618 MPa and strength 67.3 MPa), yet both broke at similar ultimate strains [15.7 and 16.3% for immature and mature, respectively (Nakagawa et al., 1996)].

Advances in ultrasound methods have made it possible to measure EM *in vivo*, and to track changes in modulus longitudinally throughout an exercise intervention. This approach has in some cases shown dramatic changes in EM, including one study that measured a 69% increase in EM in response to exercise in an elderly human population (Reeves et al., 2003). Breaking stress cannot of course be estimated *in vivo*, but if breaking stress and material stiffness are correlated, then the large change in EM observed by Reeves and co-workers (Reeves et al., 2003) would also mean a similarly large increase in breaking stress. It seems reasonable to speculate that observed changes in EM with exercise interventions may simply be secondary to an adaptive response to increase damage resistance. It has also been proposed that changes in EM with training are related to adaptive responses to reduce tendon susceptibility to fatigue damage (Buchanan and Marsh, 2001).

Physiological basis for variation in tendon properties

The mechanisms underlying variation in tendon material properties – both within an individual tendon in response to the mechanical environment, or between tendons of different muscles – are not entirely clear (Kjaer, 2004). Both the collagen fiber backbone, as well as other components of the extracellular matrix, have been proposed to contribute to variation in tendon material properties. Exercise studies in animals have demonstrated that increased tendon stiffness is associated with increased collagen density (Woo et al., 1980) and with alterations in collagen fibril crimp angle (Wood et al., 1988). Collagen turnover rates respond chronically to training (Langberg et al., 2001) and acutely to exercise (Langberg et al., 1999), along with up-regulation of other proteins and proteoglycans (Miller et al., 2005). Other factors that may contribute to the variability of tendon material properties include the presence of certain proteoglycans (Robinson et al., 2005), and changes in the chemical environment (e.g. acidity) of tendon extracellular matrix (Grant et al., 2009). Fibrocartilage is incorporated in many tendons, typically in regions of compressive load and at entheses (Benjamin and Ralphs, 1998). The tendons tested in this study wrap around the ankle joint, thus fibrocartilage elements may contribute to the observed variation in material properties. The response of various growth factors and signaling molecules to repetitive loading (reviewed by Kjaer, 2004), as well as increased tendon metabolic activity with exercise (Kalliokoski et al., 2005; Bojsen-Moller et al., 2006), further highlight the dynamic nature of this tissue.

Conclusion

Our results support the idea that material properties of tendons vary among muscles. The observed positive relationship between EM and UTS suggest that although tendon may be plastic and adaptable to varying mechanical environments, adaptive responses may ultimately be constrained by coupling between different material properties. Whether there is a structural basis for this proposed coupling remains to be determined.

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REFERENCES

Alexander, R. M. (1991). Energy-saving mechanisms in walking and running. *J. Exp. Biol.* **160**, 55-69.

Alexander, R. M. (2003). *Principles of Animal Locomotion.* Princeton, NJ: Princeton University Press.

Arruda, E. M., Calve, S., Dennis, R. G., Mundy, K. and Baar, K. (2006). Regional variation of tibialis anterior tendon mechanics is lost following denervation. *J. Appl. Physiol.* **101**, 1113-1117.

Azizi, E., Halenda, G. M. and Roberts, T. J. (2009). Mechanical properties of the gastrocnemius aponeurosis in wild turkeys. *Integr. Comp. Biol.* **49**, 51-58.

Benjamin, M. and Ralphs, J. R. (1998). Fibrocartilage in tendons and ligaments – an adaptation to compressive load. *J. Anat.* **193**, 481-94.

Bennett, M. B. (1985). Interrelationships of crural muscles and tendons in a range of birds. *J. Zool.* **235**, 33-42.

Bennett, M. B. and Stafford, J. A. (1988). Tensile properties of calcified and uncalcified avian tendons. *J. Zool.* **214**, 343-351.
Bennett, M. B., Ker, R. F., Dimery, N. J. and Alexander, R. M. (1986). Mechanical-properties of various mammalian tendons. *J. Zool.* **209**, 537-548.
Bojsen-Moller, J., Kalliokoski, K., Seppanen, M., Kjaer, M. and Magnusson, S. P. (2006). Low-intensity tensile loading increases intratendinous glucose uptake in the Achilles tendon. *J. Appl. Phys.* **101**, 196-201.
Buchanan, C. I. and Marsh, R. L. (2001). Effects of long-term exercise on the biomechanical properties of the Achilles tendon of guinea fowl. *J. Appl. Physiol.* **90**, 164-171.
Cavagna, G. A., Heglund, N. C. and Taylor, C. R. (1977). Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. *Am. J. Physiol.* **233**, R243-R261.
Chen, L. X., Wu, Y. P., Yu, J. K., Jiao, Z. D., Ao, Y. F., Yu, C. L., Wang, J. Q. and Cui, G. Q. (2011). Effect of repeated freezing-thawing on the Achilles tendon of rabbits. *Knee Surg. Sports Traum. Arthr.* **19**, 1028-1034.
Cui, L., Maas, H., Perreault, E. J. and Sandercock, T. G. (2009). *In situ* estimation of tendon material properties: Differences between muscles of the feline hindlimb. *J. Biomech.* **42**, 679-685.
Grant, C. A., Brockwell, D. J., Radford, S. E. and Thomson, N. H. (2009). Tuning the elastic modulus of hydrated collagen fibrils. *Biophys. J.* **97**, 2985-2992.
Harvey, E. B., Kaiser, H. E. and Rosenberg, L. E. (1968). An atlas of the domestic turkey (*Meleagris gallopavo*); myology and osteology. Washington, DC: US Atomic Energy Commission.
Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* **3**, 034001.
Heglund, N. C., Fedak, M. A., Taylor, C. R. and Cavagna, G. A. (1982). Energetics and mechanics of terrestrial locomotion. IV. Total mechanical energy changes as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 57-66.
Kalliokoski, K. K., Langberg, H., Ryberg, A. K., Scheede-Bergdahl, C., Doessing, S., Kjaer, A., Boushel, R. and Kjaer, M. (2005). The effect of dynamic knee-extension exercise on patellar tendon and quadriceps femoris muscle glucose uptake in humans studied by positron emission tomography. *J. Phys.* **99**, 1189-1192.
Ker, R. F. (1981). Dynamic tensile properties of the plantaris tendon of sheep (*Ovis aris*). *J. Exp. Biol.* **93**, 283-302.
Ker, R. F. (2007). Mechanics of tendon, from an engineering perspective. *Int. J. Fatigue* **29**, 1001-1009.
Ker, R. F., Alexander, R. M. and Bennett, M. B. (1988). Why are mammalian tendons so thick? *J. Zool.* **216**, 309-324.
Kjaer, M. (2004). Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Phys. Rev.* **84**, 649-698.
Konow, N., Azizi, E. and Roberts, T. J. (2011). Muscle power attenuation by tendon during energy dissipation. *Proc. Biol. Sci.* **279**, 1108-1113.
Kubo, K., Kawakami, Y., Kanehisa, H. and Fukunaga, T. (2002). Measurement of viscoelastic properties of tendon structures *in vivo*. *Scand. J. Med. Sci. Sports* **12**, 3-8.
Langberg, H., Skovgaard, D., Petersen, L. J., Bulow, J. and Kjaer, M. (1999). Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. *J. Phys.* **521**, 299-306.
Langberg, H., Rosendal, L. and Kjaer, M. (2001). Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. *J. Phys.* **534**, 297-302.
Legerlotz, K., Riley, G. P. and Screen, H. R. C. (2010). Specimen dimensions influence the measurement of material properties in tendon fascicles. *J. Biomech.* **43**, 2274-2280.
Lichtwark, G. A. and Wilson, A. M. (2006). Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J. Exp. Biol.* **209**, 4379-4388.
Lieber, R. L., Leonard, M. E., Brown, C. G. and Trestik, C. L. (1991). Frog semitendinosis tendon load-strain and stress-strain properties during passive loading. *Am. J. Phys.* **261**, C86-C92.
Maganaris, C. N. (2002). Tensile properties of *in vivo* human tendinous tissue. *J. Biomech.* **35**, 1019-1027.
Maganaris, C. N. and Paul, J. P. (2000). Hysteresis measurements in intact human tendon. *J. Biomech.* **33**, 1723-1727.
Maganaris, C. N. and Paul, J. P. (2002). Tensile properties of the *in vivo* human gastrocnemius tendon. *J. Biomech.* **35**, 1639-1646.
Maganaris, C. N., Narici, M. V. and Reeves, N. D. (2004). *In vivo* human tendon mechanical properties: effect of resistance training in old age. *J. Musculoskelet. Neuronal Interact.* **4**, 204-208.
Magnusson, S. P., Narici, M. V., Maganaris, C. N. and Kjaer, M. (2008). Human tendon behaviour and adaptation, *in vivo*. *J. Phys.* **586**, 71-81.
Matsumoto, F., Trudel, G., Uhthoff, H. K. and Backman, D. S. (2003). Mechanical effects of immobilization on the Achilles tendon. *Arch. Phys. Med. Rehabil.* **84**, 662-667.
Mendez, J. and Keys, A. (1960). Density and composition of mammalian muscle. *Metabolism* **9**, 184-188.
Miller, B. F., Olesen, J. L., Hansen, M., Døssing, S., Crameri, R. M., Welling, R. J., Langberg, H., Flyvbjerg, A., Kjaer, M., Babraj, J. A. et al. (2005). Co-ordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J. Phys.* **567**, 1021-1033.
Nakagawa, Y., Hayashi, K., Yamamoto, N. and Nagashima, K. (1996). Age-related changes in biomechanical properties of the Achilles tendon in rabbits. *Eur. J. Appl. Physiol. Occup. Physiol.* **73**, 7-10.
Narici, M. V., Maffulli, N. and Maganaris, C. N. (2008). Ageing of human muscles and tendons. *Disabil. Rehabil.* **30**, 1548-1554.
Pollock, C. M. and Shadwick, R. E. (1994). Relationship between body mass and biomechanical properties of limb tendons in adult mammals. *Am. J. Physiol.* **266**, R1016-R1021.
Reeves, N. D., Maganaris, C. N. and Narici, M. V. (2003). Effect of strength training on human patella tendon mechanical properties of older individuals. *J. Physiol.* **548**, 971-981.
Roberts, T. J. (2002). The integrated function of muscles and tendons during locomotion. *Comp. Biochem. Physiol.* **133A**, 1087-1099.
Roberts, T. J. and Azizi, E. (2011). Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *J. Exp. Biol.* **214**, 353-361.
Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113-1115.
Roberts, T. J., Chen, M. and Taylor, C. (1998). Energetics of bipedal running. II. Limb design and running mechanics. *J. Exp. Biol.* **201**, 2753-2762.
Robinson, P. S., Huang, T.-F., Kazam, E., Iozzo, R. V., Birk, D. E. and Soslowsky, L. J. (2005). Influence of decorin and biglycan on mechanical properties of multiple tendons in knockout mice. *J. Biomech. Eng.* **127**, 181-185.
Sawicki, G. S., Lewis, C. L. and Ferris, D. P. (2009). It pays to have a spring in your step. *Exerc. Sport. Sci. Rev.* **37**, 130-138.
Schatzmann, L., Brunner, P. and Stäubli, H. U. (1998). Effect of cyclic preconditioning on the tensile properties of human quadriceps tendons and patellar ligaments. *Knee Surg. Sports Traum. Arthr.* **6**, S56-S61.
Shadwick, R. E. (1990). Elastic energy storage in tendons: mechanical differences related to function and age. *J. Appl. Physiol.* **68**, 1033-1040.
Svendsen, K. H. and Thomson, G. (1984). A new clamping and stretching procedure for determination of collagen fiber stiffness and strength relations upon maturation. *J. Biomech.* **17**, 225-229.
Vanden Bergen, J. S. and Zweers, G. A. (1993). Myologia. In *Handbook of Avian Anatomy* (ed. J. J. Baumel), pp. 189-251. Cambridge, MA: Nuttall Ornithological Club.
Woo, S. L. (1982). Mechanical properties of tendons and ligaments. I. Quasi-static and non-linear viscoelastic properties. *Biorheology* **19**, 385-396.
Woo, S. L., Ritter, M. A., Amiel, D., Sanders, T. M., Gomez, M. A., Kuei, S. C., Garfin, S. R. and Akeson, W. H. (1980). The biomechanical and biochemical properties of swine tendons-long term effects of exercise on the digital extensors. *Connect Tissue Res.* **7**, 177-183.
Wood, L. K., Arruda, E. M. and Brooks, S. V. (2011). Regional stiffening with aging in tibialis anterior tendons of mice occurs independent of changes in collagen fibril morphology. *J. Appl. Phys.* **111**, 999-1006.
Wood, T. O., Cooke, P. H. and Goodship, A. E. (1988). The effect of exercise and anabolic steroids on the mechanical properties and crimp morphology of the rat tendon. *Am. J. Sports Med.* **16**, 153-158.
Zajac, F. E. (1989). Muscle and tendon: properties, models, scaling, and application to biomechanics and motor control. *Crit. Rev. Biomed. Eng.* **17**, 359-411.